

ABSTRACT

A method for *in vitro* molecular evolution of protein function

5 The invention provides a method for generating a polynucleotide sequence or population of sequences from parent single stranded polynucleotide sequences encoding one or more protein motifs, comprising the steps of

- a) providing single stranded DNA constituting plus and minus strands of parent polynucleotide sequences;
- 10 b) digesting the single stranded polynucleotide sequences with a nuclease other than DNase I to generate populations of single stranded fragments;
- c) contacting said fragments generated from the plus strands with fragments generated from the minus strands and optionally, adding primer sequences that anneal to the 3' and 5' ends of at least one of the parent polynucleotides under annealing conditions;
- 15 d) amplifying the fragments that anneal to each other to generate at least one polynucleotide sequence encoding one or more protein motifs having altered characteristics as compared to the one or more protein motifs encoded by said parent polynucleotides.